CyGEL™ in Plate-Based Cytometry

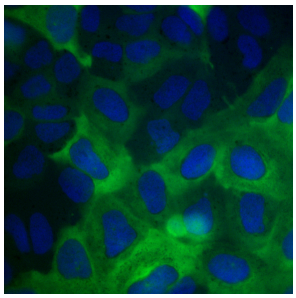
Thermoreversible Hydrogel Mountant for Non-adherent/Motile Cells

biostatus

CY1.APPNOTE: PLATE CYTOMETRY 001 130814

1. IMMOBILISING NON-ADHERENT CELLS IN MICROPLATES

BACKGROUND



For some applications flow cytometry has several shortcomings, including a relatively low throughput and frequent requirement for large numbers of cells. A more fundamental problem is the inability to analyze adherent cell lines *in situ* or 'revisit' the same cell or even the same 'neighbourhood' of a cell population. Image-based microplate cytometry offers a solution with a typical reduction in sample volume and an increase in the number of samples that can be analysed conveniently. The very nature of the microplate format allows an instrument to revisit the same well address or even a known location within a well.

WHAT IS THE PROBLEM?

The problem arises when non-adherent cells need to be used in microplate formats since they are essentially mobile within the well. Furthermore, initially adherent cells may detach from the well surface during cell division, cell death or in response to an experimental procedure. Non-adherent cells in a conventional culture medium or buffer often do not distribute evenly within the micro-well due to settling, rolling and clumping artifacts made worse by the 'panning' effects of movements of the microplate. Frequently non-adherent cells will concentrate in the centre or at the perimeter of the well. To overcome these issues suspension cells can be centrifuged onto the well surface and fixed or 'tacked' to the surface using adhesion reagents – all potentially introducing bias in the cells that are immobilized. An alternative provided by CyGEL™ is to capture *all* cells in an immobilizing, optically compatible hydrogel.

HOW DOES CyGEL™ HELP?

CyGEL™ allows you to observe live cells in real-time experiments without worrying about their motility or movement. CyGEL™ works as a thermoreversible hydrogel - *liquid when cold and a gel when warmed*. It is compatible with LIVE cells suspensions. It is designed as a flexible general purpose immoblising medium for the capture and analysis of suspension cell populations in microplates and not as a long-term growth matrix. The gel is formulated to be liquid when cool so can be conveniently stored at 4 deg C and mixed with a cell preparation. Upon warming beyond room temperature to 37 deg C, a CyGEL™ preparation rapidly transitions to a gel that can be returned within seconds to a liquid by simple cooling. CyGEL™ gels can also be slowly dissolved by the addition of chilled excess buffer. This allows the operator to generate simple protocols for the capture, analysis and indeed the recovery of cell populations. CyGEL™ is compatible with culture media, small molecules such as viability dyes, vital dyes and anaesthetics, fluorescence and standard microscopy optics.

CyGEL™ allows you to explore different microplate-based applications including incorporating evenly distributed reference beads into your samples for simple controls or 'in sample' calibrations. You can exploit the fact that cells within a hydrogel experience reduced diffusion rates for permeant molecules across the plasma membrane - both into and out of the cell. For example, viable cell DNA dyes such as DRAQ5™ when incorporated into CyGEL™ enter cells at reduced diffusion controlled rates, stretching the difference between rapidly-staining dead cells and slow-staining viable cells. Alternatively, a gel 'overlay' for adherent cells in a micro-well will reduce the loss of reporter molecules – extending the analysis window for processing in a live cell microplate assay.

CyGEL™ Product Features:

- convenient immobilisation of non-adherent cells and beads
- optically clear with low autofluorescence for visible range excitation
- controllable performance and rapid reversible transition from liquid to gel
- compatible with GFP and "in gel" fluorescent probes including DRAQ5™

For a full price list and further information see **www.biostatus.com** or contact us at:
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